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1: Biotechniques 1996 Feb;20(2):219-25 Related Articles, Links

Evaluation of different amplification protocols for use in primer-extension preamplification.

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Different amplification protocols were evaluated for use with primer-extension preamplification (PEP). We hypothesized that a protocol known to improve amplification of long DNA fragments would improve efficacy of PEP. Eight DNA samples were preamplified by PCR using different protocols. Treatments consisted of the use of Taq DNA polymerase (T), Taq plus a second polymerase obtained from Pyrococcus furiosus (E) or Stoffel fragment (S) in PEP. After preamplification, six genetic markers were genotyped, and the number of scorable genotypes was recorded. A control reaction (C) consisted of amplification using genomic DNA as template. A second experiment was performed to evaluate preamplification efficiency using Taq DNA polymerase (5 units) and exponential dilutions of Pfu DNA polymerase. After preamplification, the same procedure was used to obtain a number of scorable genotypes. In the first experiment, treatment E was the most reliable approach for amplifying genomic DNA in PEP. Treatments T and S produced fewer scorable genotypes than treatments E or C. In the second experiment, low concentrations of Pfu DNA polymerase produced a similar percentage of scorable genotypes as higher concentrations. Low concentrations of Pfu DNA polymerase combined with Taq DNA polymerase is the most cost-effective procedure to maximize amplification of limited DNA samples in PEP.

PMID: 8825151 [PubMed - indexed for MEDLINE]

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Whole genome amplification of single cells: mathematical analysis of PEP and tagged PCR.

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We construct a mathematical model for two whole genome amplification strategies, primer extension preamplification (PEP) and tagged polymerase chain reaction (tagged PCR). An explicit formula for the expected target yield of PEP is obtained. The distribution of the target yield and the coverage properties of these two strategies are studied by simulations. From our studies we find that polymerase with high processivity may increase the efficiency of PEP and tagged PCR.

PMID: 7659528 [PubMed - indexed for MEDLINE]

1: Hum Genet 1996 May;97(5):591-5

Human Genetics

Reevaluation of the exact CAG repeat length in hereditary cerebellar ataxias using highly denaturing conditions and long PCR.

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Hereditary cerebellar ataxias, including spinocerebellar ataxia type I (SCA1), dentato-rubro-pallidoluysian atrophy (DRPLA), and Machado-Joseph disease (MJD), have been associated with unstable CAG repeats. The length of the CAG repeat is a major factor in determining the age of onset of these diseases. In electrophoresis through acrylamide gels with formamide, the CAG repeat length following the polymerase chain reaction (PCR) coincides with the sequence-determined repeat length after subcloning. However, without formamide, PCR products with long CAG repeats appear 1-4 repeats shorter than when electrophoresed with formamide, and the repeat lengths are variable. In addition, the larger the CAG repeats are, the more difficult are the PCR reactions. A mixture containing thermostable Taq and Pwo DNA polymerases (so-called "long PCR") is much more sensitive than that with Taq polymerase alone in detecting- expanded CAG repeats. Therefore, highly denaturing conditions, especially formamide gel electrophoresis, and the "long PCR" protocol should be used to evaluate the exact CAG repeat length. We have used these principles to detect unstable CAG repeats. The normal ranges are 14-34 repeats for MJD, 6-31 repeats for DRPLA, and 21-32 repeats for SCA1.

PMID: 8655136 [PubMed - indexed for MEDLINE]

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